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BACTERIAL ROT OF STORED POTATO TUBERS

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BY

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The successful preservation of potato tubers in store is a problem of importance in India owing to the increase in the use of this form of food by natives in various provinces. The introduction and spread of the potato moth has made the question of successful storage more difficult than formerly, and it is complicated by the liability to rotting produced by various bacteria and fungi commonly present in the soil in which the potatoes are grown, or in that used to cover and protect the tubers from the attacks of the potato moth in store. It is not proposed in this paper to describe either insect or fungus sources of damage, but only to refer to them in connection with those of bacterial origin to which they may be contributory, or to do so in recommending preventive measures intended to apply equally to all three. The importance of the inquiry may be gauged by the fact that samples of potatoes which had rotted in store have been received from very numerous localities, including such widely separated districts as Poona, Cawnpore, Sabour (Bihar), and Shillong, and in all of these, cases of bacterial infection were found produced by organisms which there is every reason to believe are common soil bacteria in India although they have not been described as causing potato or other rot in other countries.

In April 1913 diseased potato tubers were received at Pusa from Sabour and other places in Bihar and from Poona in the Bombay Presidency. The tubers received were in different stages of rotting. Minute black specks on the outside surface were the only signs of rot in the first stage, in which

the tuber was found sound inside. In later stages these black marks extended through the skin to the flesh to varying depths. The most advanced stage was marked by large discoloured patches of loose skin, covering cavities containing gas ; greyish white frothy matter oozed out from these discoloured patches in some cases. Transverse sections showed brownish discoloration extending to varying depths, originating in all cases from the discoloured portion in the surface layer. The yellowish white or greyish matter exuding from the darkened portions was of a slimy consistency and under the microscope was found to be full of bacteria of varying morphological characters.

In Bihar the disease was first noticed in the year 1912 at Purnea, where the demonstration of storing potatoes in sand against the attack of potato moth (*Phthorimœa operculella*) failed owing to rotting of the stored potatoes. In Poona, however, the disease was noticed in 1913 at the time of digging the crop, nearly 20 per cent. decaying within two weeks of harvesting. Thus in both cases the rot originated when the potato tubers were in contact with soil, as at Poona, or with sand, as in Bihar ; in the latter case, however, in the majority of instances it is probable that the tubers were stored in sandy soil and not in pure sand ; this would considerably affect the moisture conditions during the monsoon. The symptoms of rot noted above differ from those associated with *B. solanacearum* in the fact that disintegration and discoloration of the tissues in the former case originate at the surface and involve the whole tuber, whereas in the case of the latter the attack generally originates within the vascular bundles, to which it is at first confined, and spreads much more slowly.

Six varieties of potato tubers were received from different localities in Bihar and Poona and in all of these rotting was found.

Darjeeling variety.
Naini Tal.
Fulwalla.
Local Red (Bihar).
Local White (Bihar).
Potatoes from Poona.

In addition to the rotting potatoes examined in the year 1913, local potatoes which were rotting in the laboratory were examined, as well as those that were sent from Cawnpore and from Bihar in August 1914. The signs of the disease were the same as before.

Bacterial cultures were made on agar from several of the diseased tubers in different stages of rot, and some pure strains were isolated which were subsequently used for inoculation into sound tubers.

As seen under the microscope, large numbers of different forms of bacteria were observed in the rotting potatoes. All of these forms need not necessarily be parasitic, since it is probable that many saprophytic forms would enter after the way is prepared for them by the specific parasites. In order to eliminate such saprophytes as far as possible, sound potato tubers were inoculated with slimy material from the rotting potatoes and kept in a covered dish with some sterile water. The method of sterilizing the outside was as follows :—

The tubers were first washed thoroughly with water and then with mercuric chloride solution (1 : 1000). After being immersed in this for 15 minutes they were then washed with sterilized water and next with alcohol and were flamed on the outside. When the rot had set in in potatoes thus inoculated, which generally occurred in about 24 hours after inoculation, frothy slime exuding from the point of inoculation was used for inoculating fresh sound tubers. By repeating this process of successively inoculating sound tubers four or five times, most of the saprophytes were eliminated and it was easy to separate the parasitic forms by plating from the last rotting potato.

As observed above, the development of gas in cavities under the skin in advanced stages was a characteristic symptom, prominent by its constancy, and hence attention was directed to find out and isolate any organism derived from rotting tubers that produces gas in glucose bouillon. It was not at first suspected that any of the gas-producing organisms would be rot-producers, but merely saprophytes taking advantage of the previous entry and enzymic activity of the specific parasitic organism ; purely saprophytic organisms usually require excess of water or anaerobic conditions to produce rot in potato tubers together with the butyric acid formation characteristic of this form of attack, but at the time when gas has accumulated under the skin it is very likely that the gas-producing organism may be in predominance and thus suppress the specific rot-producer ; and so an attempt to separate the gas-producing organism was chiefly made with a view to distinguish it from the rot-producer and prevent confusion of one with the other. This was accomplished by inoculating slimy material from a rotting potato into glucose bouillon and by successive transfers and plating on agar. From the last transfer a pure culture of the gas-producing organism was obtained.

Out of the several forms isolated by these methods from the rotting potatoes received in the year 1913, two organisms were found which on inoculation rapidly produce the definite symptoms of rot noted above in sound tubers.

From rotting potatoes examined in the year 1914 two other organisms were isolated which were found to cause rot when inoculated in sound potatoes.

It may be noted here that of these four organisms the one described hereafter as No. 1 was noticed most frequently as occurring in rotting potatoes from all localities. The second organism described as No. 2 occurs less frequently. The one described as No. 3 resembles *B. coli* and was found in rotting potato tubers from Pusa and Cawnpore. Organism No. 4 though occurring but rarely is described here chiefly on account of its resemblance to *B. xanthochlorum* (Schuster) and to *B. pyocyaneus.* All the strains of the organism No. 4 did not invariably cause rot, which may be due to variation in virulence.

That there is more than one organism which by itself may cause rotting of potatoes is shown by the fact that several authors have found a different organism connected with the rot. Schuster[1] who has worked on four different types along with *B. xanthochlorum* has also stated that important differences in the physiological activities of the organisms causing potato-rot exist.

Plate cultures demonstrated the presence amongst others of the following saprophytic forms, *viz., B. subtilis, B. mesentericus, B. mycoides, B. pyocyaneus,* and *B. prodigiosus.* Many others may have escaped notice owing to the fact that attention was soon directed to the rot-producers. It has been demonstrated by some workers that some of the saprophytes mentioned above developed into active parasites producing potato rot. Thus Schuster mentions that Laurent[2] and Lepoutre[3] obtained strains of *B. fluorescens liquefaciens, B. mesentericus* and *B. mycoides* as virulent parasites by cultural methods. No such result was obtained here with any of the organisms isolated, excepting with the four about to be described.

The following are the physiological and cultural characteristics of the four organisms isolated and found capable of producing rot when inoculated into sound tubers.

ORGANISM No. I.

Isolated from Rotting Potatoes from Bihar and Orissa.

Motile. Peritrichic flagella.

$0{\cdot}5\mu \times 1{\cdot}2\mu$ to $1{\cdot}6\mu$.

Ordinary agar + 5 Fuller's Scale (at 30°C.)

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1 Schuster, *Arbeit. Kais. Biol. Anst. f. Land-u. Forstwirtsch.* VIII, 4, **1912.**
2 Laurent, Emile. *Ann. Inst. Pasteur*, Vol. XIII, pp. 1-48, 1899.
3 Lepoutre, M. L. *Ann. Inst. Pasteur.* Vol. XVI., pp. 304-312, 1902.

Agar Plate Colonies.—White, opalescent, nucleated or non-nucleated colonies. Moist, shining, brownish, and granular under the microscope. Translucent to transparent; slightly raised. When older the colonies become yellowish grey.

Agar Streak.—Growth abundant; white, opalescent, moist, shining, raised; white precipitate in condensation water; as the growth gets older it becomes yellowish in the centre.

Gelatine stab (at 22°C.)—Does not liquefy; grows on the surface. After a few successive generations on agar however, it acquires the property of liquefying gelatine, while at the same time its action on potato becomes slower.

Bouillon + 5 F.—Thin pellicle, granular precipitate, Indol produced. Lead acetate paper (held by the plug) blackened in 24 hours, showing production of H_2S.

Peptone water + 5 F.—Thin pellicle at the surface: granular white precipitate at the bottom; cloudy; Indol formed.

Glucose peptone water + 5 F.—Gas produced. Acid reaction at the end of 10 days. Granular precipitate at the bottom. Medium cloudy.

Lactose peptone water + 5 F.—Gas and acid produced. Granular precipitate at the bottom at the end of 10 days. Medium cloudy.

Saccharose peptone water + 5 F.—Gas and acid produc d. Granular precipitate at the bottom at the end of 10 days. Medium cloudy.

Glycerine peptone water + 5 F.—Granular precipitate at the bottom of tube.

Maltose peptone water.—Acid and gas produced; medium cloudy, and precipitate at the bottom.

Litmus peptone water.—Litmus reduced.

Methylene-blue peptone water.—Methylene-blue reduced.

Nitrate broth.—Nitrates reduced without any gas formation.

Uschinsky's solution.—Medium cloudy within 24 hours, precipitate at the bottom, and a thin pellicle.

Litmus milk.—Litmus reddened: milk slightly coagulated and repeptonized after a week.

Potato steamed.—Growth yellowish-white, moist, glistening, later brownish, spreading slightly. Potato water cloudy after 2 days. Alkaline to litmus, alcohol present (iodoform test).

Starch agar.—Opalescent growth with precipitate in the condensation water. Starch reaction with iodine disappears completely after 2 days.

Inoculated into a medium of the following composition:—

K_2HPO_4	0·5 gm.
$MgSO_4$	0·5 ,,
NaCl	0·5 ,,
Amm. sulphate	1·0 ,,
$CaCO_3$	1·0 ,,
Potato starch	5·0 gms.
Water	1000 c.c.

It grew very well and formed sugar as tested by Fehling's solution, and alcohol as tested by iodoform.

Aerobism.—Agar and bouillon tubes inoculated with the organism were kept in jars from which air was exhausted under the pump, the remaining oxygen being absorbed by caustic potash and pyrogallic acid. The bouillon tubes clouded, and agar streaks grew just as well as those kept in the incubator under aerobic conditions.

Optimum temperature.—The organism grows at temperatures varying from 20° to 37°C. A comparison of bouillon tubes, agar streaks, and Uschinsky's solution at temperatures varying between these limits showed 30°C. to be the optimum for this organism.

Thermal death point.—To determine the thermal death point of this organism, fresh bouillon cultures were made from a 24 hours old bouillon culture ; each culture consisting of 10 c.c. of broth was inoculated with a 2 mm. loop from the 24 hours old culture. These freshly inoculated tubes were placed in a water bath at temperatures of 40°, 45°, 50°, 55° and 60°C. for 10 minutes respectively. Next day there was growth in the tube heated to 45°C. After 48 hours there appeared some growth at 50°C. but none appeared in tubes heated to 55°C. and 60°C. The experiment was repeated at temperatures between 50° and 55°C. Growth took place in tubes heated to 51°, 52°, 53° but none appeared in tubes heated to 54°C. and 55°C. 54°C. is therefore the thermal death point.

Direct sunlight.—To determine the effect of direct sunlight upon the organism, five tubes each containing 10 c.c. agar were inoculated and poured into thin Petri dishes, one half of each dish was covered with black paper, and the dishes, after the agar solidifying, were kept on a plate cooled by ice and exposed to direct sunlight for 5, 10, 15, 30 and 60 minutes respectively. There was no effect observable in the plates exposed upto 30 minutes. Those that

were exposed for 60 minutes showed about 10% decrease in colonies in the exposed part.

Diffused light.—Diffused light had no effect upon the development of the organism.

Inoculations into raw potatoes :—

The potato tubers were first immersed for 15 minutes in corrosive-sublimate solution (1 : 1000) ; washed with sterile water and then with alcohol ; after flaming they were placed in a sterilized dish with a small quantity of sterile water. The tubers were next punctured with a hot sterile needle and inoculated with a 24 hours old culture of the organism. There were control tubers similarly treated but without inoculation. Rotting set in 48 hours after inoculation. The visible signs were (*a*) bubbles of gas, (*b*) slimy material oozing out from the punctures. After four or five days each potato tuber was more or less completely destroyed, practically nothing being left except the skin. The control tubers remained intact. Occasionally only (in about 5% cases) some fungus growth was noticed in the control tubers, but no rot. Plating on agar from the rotting potato tubers thus inoculated gave the organisms again, and very rarely was there any contaminating organism. Sugar was found in the rotting tubers in the early stages of decay in two to four days, but disappeared later when acid and gas production had become pronounced ; in a later stage the acid reaction was replaced by an alkaline one.

Rotting was also produced when cultures of this No. 1 organism were smeared on the unbroken outer surface of the tubers ; the conditions under which such rotting took place are described later in this paper.

Inoculations into other edible tubers and roots :—

Inoculations were made into

Suthni (*Dioscorea* sp.)—
Arva (*Colocasia antiquorum*),
Sweet Potato (*Ipomœa Batatas*),
Onions (*Allium Cepa*), and
Carrots (*Daucus Carota*)

to see whether these are attacked by the bacterium when inoculated. Inoculations were successful in *suthni* and sweet potato after a long time. In onion and *arva* it failed to produce any rot. The results of inoculations into carrot were not conclusive since the control ones also rotted.

Enzymes.—The enzymes of this organism were secured from bouillon culture and the starch solution medium noted above, by precipitating with

diastasic power through disuse. Such organisms as these would be valuable subjects for investigation along the lines of research in which attempts have recently been made to demonstrate the possibility of inducing permanent artificial variation in type amongst schizomycetes.

In this connection it is interesting to compare the group numbers of various other rot-producing organisms.

221·3333013	*B. phytophthorus* (Appel). Potato tuber and stem rot.
221·3333113	*B. xanthochlorum* (Schuster). Potato tuber and stem rot.
221·1110	Potato Black-stalk *B. melanogenes* (Pethybridge and Murphy). Potato plant disease.
121·111352	*B. tubifex* (Dale). Potato leaf disease.
222·1113523	*B. apiovorus* (Celery Rot).
221·1113022	*B. aroidae* (Calla Lilly Rot).
221·1113022	*B. omnivorus* (Iris Rot).
221·2223022	Kale Rot.
221·2123022	Turnip Rot (Potter's Bacillus).
221·1113022	Cauliflower Rot (Harrison).

It may be pointed out that the chief variations in the group number result from differences in fermenting power for various sugars, as has also been pointed out by Hardinge and Morse[1] in their study of soft rot organisms.

ORGANISM No. II.

Isolated from Rotting Potatoes from Bihar and Poona.

Non-motile. No flagella.

$0{\cdot}8\mu$ to $1{\cdot}0\mu \times 1{\cdot}6\mu$ to $2{\cdot}0\mu$

Ordinary agar + 5 F.

Colonies.—Nucleated with a halo round the nucleus. Yellow nucleus and greenish yellow halo. Edges slightly thickened. Opaque in the centre, translucent at the edges, moist at first, dry after 48 hours. Dull lustre.

Agar streak.—Yellow growth with a halo at the sides. Yellow precipitate in the water of condensation. Dry, flat, slightly raised in the centre. No change in the colour of medium.

Gelatine + 5 F. (at 22°C).—Does not liquefy gelatine ; surface growth. Moist, raised, yellow, shining.

Bouillon + 5 F.—Slight granular yellow precipitate at the bottom and a thin ring at the side of the surface. Cloudy at first, clearing after 3 or 4 days. No H_2S evolved as tested by lead acetate paper. Indol not produced.

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[1] Hardinge & Morse, *Tech. Bull. No.* 11, *New York Expt. Sta.*, 1909.

Peptone water + 5 F.—Slight granular yellow precipitate at the bottom and a thin ring at the sides of the surface. Cloudy at first, clearing after 3 or 4 days. No indol formation.

Glucose peptone water + 5 F.—No gas. Precipitate at the bottom; slightly acid after seven days. Medium clears after 3 or 4 days.

Lactose peptone water + 5 F.—No gas; precipitate at the bottom. Slightly alkaline reaction. Medium clears after 3 or 4 days.

Saccharose peptone water + 5 F.—No gas; precipitate at the bottom. Slightly acid reaction. Medium clears after 3 or 4 days.

Glycerine peptone water + 5 F.—No gas; precipitate at the bottom. Slightly acid reaction.

Maltose peptone water + 5 F.—No gas; slightly alkaline reaction, precipitate at the bottom.

Litmus peptone water.—Litmus not reduced.

Methylene-blue peptone water.—Methylene-blue not reduced.

Nitrate broth.—No gas; precipitate at the bottom. Nitrate reduced to nitrites and ammonia.

Uschinsky's solution.—Slightly cloudy; no pellicle, slight precipitate at the bottom.

Litmus milk.—Slight yellow precipitate at the bottom, milk repeptonized. Litmus changed to slightly blue at the top.

Potato.—Yellow dry flat growth at first, becoming moist after 4 or 5 days. Water clear, with a slight film at the top. Reaction of water alkaline to litmus. No alcohol present.

Starch agar.—Yellowish, non-spreading growth: precipitate in the condensation water. Starch does not disappear completely in 48 hours.

Starch solution.—Poor growth. No sugar formed.

Starch solution + phosphates, etc. (For composition of solution see previous page).—Produces sugar. No alcohol.

Aerobism.—Facultative anaerobic.

Optimum temperature.—Grows well from 20° to 35°C.

Thermal death point.—This was determined as in the case of previous organism. The organism does not survive 57·5°C.

Direct sunlight.—This was determined as in the previous case by exposing freshly inoculated Petri dishes which were kept on a plate cooled by ice for 5, 10, 15, 30 and 60 minutes.

Exposed to direct sun.

5 minutes.	No appreciable effect.			
10	,,	10%	organisms	killed.
15	,,	30%	,,	,,
30	,,	90%	,,	,,
60	,,	100%	,,	,, there

being no growth in the Petri dish.

Diffused light.—Checks the growth of this organism.

Inoculations into raw potatoes.—Organism No. 2 was rather slower than No. 1 in producing rot in raw tubers, no symptoms being visible until after 3 or 4 days. These symptoms were similar to those observed in the case of No. 1 ; including the production of sugar, followed by acid and gas and subsequent alkaline reaction.

Enzymes.—

Diastase.—This organism produces diastase as seen by its action on starch solution + phosphate, transforming starch into sugar.

Cytase.—Tested by its action on thin razor sections of potato in bouillon cultures (*a*) heated to 65°C, and (*b*) not heated. Cell walls were also found to separate easily after about 4 or 5 days incubation. The action was slower than in the case of organism previously described as No. 1.

Group Number 222·3333523.

It will be seen that there are characteristic differences between these two organisms, the chief among these being the following :—

(1) Gas produced from all kinds of sugar by No. 1 while gas is not produced from any of the sugars by No. 2.

(2) In *Litmus Milk* No. 1 produces an acid and No. 2 an alkaline reaction.

(3) No. 1 is motile and flagellated and No. 2 non-motile and non-flagellated.

(4) No. 1 acquires the power of liquefying gelatine by cultivation on agar, No. 2 does not.

Both, however, possess the power of secreting diastase and converting starch into sugar.

SLIMY WHITE ORGANISM No. III.

Isolated from Rotting Potatoes from Pusa and Cawnpore.

Non-motile. No Flagella.

1·6μ × 1·0μ to 1·2μ ; occurs in short chains of two to four. Gram negative. Spores not found.

Ordinary agar + 5 F. at 30°C.

Colonies.—Colourless or snow-white. Transparent, *slimy*, viscous, with round edges, raised in the centre ; moist, shining.

Streak.—White growth ; thin pellicle on the condensation water.

Gelatine + 5 F., Stab (at 20° C).—Not liquefied. White button-like growth on the top.

Bouillon.—Cloudy with thin flakes at the top which sink down. Alkaline reaction. H_2S produced (shown by blackening of moist lead acetate paper held by the cotton wool plug).

Peptone water.—Cloudy at the bottom. Indol produced.

Glucose peptone water + 5 F.—Cloudy ; gas and acid produced ; precipitate ; no pellicle.

Saccharose peptone water + 5 F.—Slightly cloudy. Gas and acid produced.

Lactose peptone water + 5 F.—Cloudy. Gas and acid produced ; precipitate at the bottom.

Glycerine peptone water + 5 F.—Gas and acid produced. Medium cloudy ; precipitate at the bottom.

Maltose peptone water + 5 F.—Cloudy, precipitate at the bottom. Gas and acid produced.

Nitrate broth.—No gas. Reduced to nitrites and ammonia.

Litmus milk.—Litmus reddened after 3 or 4 days ; milk coagulated and peptonized, beginning at the top. Acid produced.

Potato.—White, moist, glistening, slightly raised. Potato-water cloudy.

Starch solution.—Poor growth.

Starch solution + phosphates, etc., solution used for showing diastasic action of Organism No. 1.—Sugar and alcohol produced ; medium acid to litmus.

Uschinsky's solution.—Cloudy ; heavy precipitate ; no pellicle.

Litmus peptone water.—Litmus reduced slightly.

Methylene-blue peptone water.—Methylene-blue reduced at the bottom.

Aerobism.—Facultative anaerobic.

Optimum temperature.—Grows well at temperatures from 20°C. to 35°C.

Thermal death point.—Determined as above described. The organism does not survive 59°C.

Direct sunlight.—This was determined as in the previous case by exposing freshly inoculated plates for 5, 10, 15, 30 and 60 minutes to direct sunlight.

5	minutes.	No effect.
10	,,	No effect.
15	,,	10% organisms killed.
30	,,	10% to 15% killed.
60	,,	70% organisms killed.

Diffused light.—Diffused light has no very marked effect.

Inoculation into raw potatoes :—

Organism No. 3 produced rot in raw tubers when inoculated into them, but not by smearing the potato tubers ; the symptoms were similar to those previously noted.

Enzymes.—Produces diastase as seen by its producing sugar and alcohol from starch solution + phosphates, etc.

Cytase.—Tested by its action on razor sections of potato in bouillon cultures (*a*) heated to 65°C., and (*b*) not heated. Action on cell walls similar to that of organism No. 1. Middle lamella was seen to be dissolved.

Group number 222·1113021.

This organism differs from organism No. 1 described previously in producing gas from glycerine and giving slimy growth. It is not motile. Flagella not demonstrated. Its breadth is comparatively great.

In order to determine whether this organism was similar to *B. coli*, as its characteristics agree with those of the latter, it was also tried on several media.

Medium.	*Kind of growth.*
Litmus glucose peptone water.	Reddened litmus, gas produced.
Neutral-red peptone water.	No change.
Neutral-red glucose peptone water.	Slightly changed its tint but no fluorescence.
Neutral-red McKonkey's bile salt agar.	Slimy growth. Slightly reddish.
Endo's fuchsin agar.	No red colonies.
Malachite-green gelatine.	Did not grow.
Litmus lactose nutrose agar.	No reddening.

This organism agrees with *B. coli* on four out of seven special media tried; on the other hand this has not shown any flagella and produces a very slimy growth.

GREENISH FLUORESCENT ORGANISM No. IV.

Resembling *B. pyocyaneus* or *B. xanthochlorum* (Schuster).

Motile. Flagella.

$1{\cdot}4\mu$ to $1{\cdot}8\mu \times 0{\cdot}4\mu$ to $0{\cdot}5\mu$ 1 or 2 flagella at one pole. Gram positive.

Ordinary agar + 5 F. at 30°C.—

Colonies.—Bluish, greenish-white; at first diffusing green pigment into the medium; as seen under low power, granular. Moist, glistening, slightly raised.

Streak.—Growth bluish-white with diffusion of green pigment into the medium; yellowish precipitate in condensation water.

Gelatine + 5 F. at 20°C.—Liquefies gelatine within 24 hours; produces yellowish and greenish pigment; reddish brown precipitate at the bottom of liquefied medium.

Bouillon + 5 F.—A greenish coloured ring formed at the top. When shaken, the colour diffuses through the medium, which is cloudy, with precipitate at the bottom, and a pellicle at the top. Alkaline to litmus. Produces NH_3 and H_2S. The pigment is decolourized by acid.

Glucose, Saccharose, Lactose, Maltose and Glycerine Peptone Water.—No gas or acid produced in any of these media.

Litmus milk.—Peptonized after coagulation; produces yellow pigment with a greenish ring at the top.

Potato.—Reddish brown growth. Greenish pigment in the potato.

Uschinsky's solution.—Medium cloudy; precipitate at the bottom with a pellicle at the top. Slight greenish pigment produced.

Nitrate broth.—Nitrates reduced to nitrites and ammonia.

Litmus peptone water.—Litmus reduced at the bottom.

Methylene-blue peptone water.—Methylene-blue reduced slightly.

Starch solution.—Poor growth.

Starch solution + phosphates, etc.—Diastasic action noticed. Sugar was formed but no alcohol. Medium became alkaline to litmus.

Aerobism.—Facultative anaerobic.

Optimum temperature.—Comparison of growth at different temperatures in agar, bouillon and Uschinsky's solution showed 37°C. as optimum.

Thermal death point.—The thermal death point of the organism was found to be 59·5°C.

Direct sunlight.—Even 5 minutes exposure was sufficient to kill about 40% of the organisms.

10 minutes	about	60%	organisms	killed.
15 ,,	,,	90%	,,	,,
30 ,,	,,	100%	,,	,,

Diffused light.—Diffused light checks the growth of this organism to a certain extent.

Inoculation into raw potatoes :—

The organism No. 4 in some cases produced rot in raw tubers when inoculated. Inoculations from very few strains are successful. Apparently similar organisms fail to produce rot. This may be supposed to be due to the variation in virulence to which this organism is subject ; other alternative suppositions are discussed by Schuster in his comparison of *B. xanthochlorum* and *B. fluorescens liquefaciens*.

Enzymes.—

Diastase.—This organism produces diastase as seen by its transforming starch into sugar in the starch solution + phosphates.

Cytase.—Tested by its action on razor sections of potato in bouillon cultures (*a*) heated to 65°C., and (*b*) not heated. Middle lamella was not seen to be acted on until after one week's incubation.

Group No. 221·3333113.

This agrees entirely with the group number of Schuster's *B. xanthochlorum.*

Mode of Infection.

On examination of the diseased potatoes originally received there was nothing to show in what manner entry of the bacteria into the tubers had been effected. The places where the black marks or gas were noticeable were not necessarily the eyes of the tubers. Samples submitted for entomological enquiry did not lead to any results, as no signs of any insect attack were noticeable on the rotting potatoes except that the skin of the tuber happened to be pierced through at the places where there were black marks.

The susceptibility of tubers to bacterial rot was tested as follows :—

Sound tubers (Naini Tal variety) were externally sterilized in the manner above described. They were divided into two lots, A and B. Those in A were punctured with a sterile needle. Both lots were kept in contact with rotting tubers for 24 hours. The unpunctured tubers in lot B were sub-

divided into two lots B_1 and B_2 of which those in B_1 were kept dry and in B_2 kept moist. The punctured tubers were similarly subdivided and treated, A_1 being kept dry and A_2 moist. All four lots were incubated at 30°C. After 24 hours the following results were noted :—

B. Unpunctured	B_1.	(Dry). Remained sound.
	B_2.	(Moist). Rotted. (Slime and gas).
A. Punctured	A_1.	(Dry). Rotted.
	A_2.	(Moist). Rotted. (Slime and gas).

Moisture or mechanical injury thus appear to be the predisposing causes of this rot. That injury whether by insect or fungus will expose the potato to attack by these four forms of bacteria is but natural, considering the diastasic power of all these organisms ; but that it is possible for these organisms without any such injury to enter the tuber and cause the rot, can be explained by the assumption that either the bacteria can attack the skin and thus enter through it or that they find entrance through some points without doing any injury to the skin. Now the tubers inoculated in the laboratory and those originally received did not show any signs of damage to the skin at any other place except where they were punctured artificially or by some natural agent, showing thereby that in all probability the rot bacteria have not the power to pierce the skin or damage it. It naturally follows then that there is some other means for the bacteria to enter the potato tuber. F. Stoward[1] in his article on the Potato Tuber describes and illustrates by means of figures the anatomy of the eyes and skin of the tuber, and concludes from his experiments that there is structurally a marked difference between the external layer of the eye of the tuber and that of the skin. This difference extends also to the permeability of the two to substances in solution ; and probably to the soluble products or ferments of the rot bacteria (such as diastase) which find first an easy entrance to the flesh of the tuber through the eyes ; and by weakening the tuber at those places create conditions which ensure a free entrance of the bacteria themselves.

The following experiment shows that this is probably the case and also that the bacteria soon find their way into the tuber. Tubers which were just sprouting were taken for this purpose in one lot and compared with those which had not begun to germinate. All these were sterilized as before, in this case without washing with alcohol and without flaming, as it was likely

1 F. Stoward, The Potato Tuber, *Journal Nat. Hist. Sci. Socy. of West Australia*, Vol. IV.

that flaming might injure the sprouts; some small quantity of emulsion of one of the rotting bacteria was poured over the potatoes by means of a sterile pipette. Sprouting potatoes were chosen because it was easier to examine the cross sections of the sprouts than of the eyes.

The result after 24 hours was that the sprouts of germinating tubers darkened, and showed bacteria when sections were examined under the microscope, within 48 hours, and the sprouting tubers began to show signs of rot within 3 days and in a cross section showed the rot originating from the place where the sprouts were situated. Tubers which had not begun to sprout showed signs of rot on the 4th or 5th day, *i.e.*, one or two days later than the germinating potatoes, but the point of entry originated where the eyes were situated.

Although the rot bacteria attack uninjured potatoes when the emulsion is smeared on the tubers, the rot generally does not appear to set in in sound potatoes under natural conditions. This may possibly be due to the fact that under such conditions the bacteria are not present in sufficient numbers, or that their virulence is not developed to a sufficient degree except when the resistance of the tuber is weakened by being first damaged by insect or fungus attack. Many diseased potatoes, when examined, presented an appearance as if their skin had been pierced through by some insect though it left no other traces behind. That this is possible may be judged by the fact that a new Tingid[1] bug was noticed to be present in godowns for storing potatoes, attacking the tubers and doing damage by sucking juice from them. The punctures made by these or other bugs probably afford points of entry to the rot bacteria.

Rhizoctonia and *Fusarium* have been found on tubers from Sabour, Cawnpore, and on local Pusa potatoes; it is probable that damage done by such fungi may lead to subsequent attack by rot-producing bacteria.

The occurrence of infection in all the different varieties received from the several godowns in Bihar and from Poona and Cawnpore suggests the presence of the rot-producing organisms as normal in soil carrying a potato crop.

This was tested in two ways:—

(i) Organisms which showed similar fermenting powers were isolated from the soil and inoculated into raw potatoes. Organisms similar to organism No. 1 were obtained and inoculated. These produced rot in about a week's time.

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1 *Journal of Agriculture, Bihar & Orissa*, Vol. I, No. 2, Oct. 1913.

(ii) Soil emulsion was inoculated into punctured potatoes. This also produced rot, but as there were fungi also growing, the rotting potatoes were plated. Bacteria similar to bacterium No. 1 were isolated and when tested by their fermenting powers on sugars were found to be identical with the latter. Infection can therefore take place from soil if the potato happens to be injured, and the organisms causing potato rot are probably present in most Indian soils.

Remedial and Preventive Measures.

Whether these organisms are introduced into the soil by the seed or not, does not come into consideration for preventing rotting in store, but assuming their universal presence in the soil as probable, it is necessary to devise some method of sterilizing the outside of the tuber before storing.

With this object in view, sound potato tubers were chosen and divided into three lots of twenty tubers each. One lot was immersed in mercuric chloride solution (1 : 1000) for 15 minutes. These tubers were allowed to dry on a porcelain plate. A second lot was immersed in formalin solution (1% formaldehyde) for 15 minutes and then put on a porcelain plate to dry. The third lot was subdivided into two lots, 3a and 3b ; tubers in 3a were immersed in 2% copper sulphate solution and those in 3b were immersed in 1% copper sulphate solution, each being kept there for 30 minutes and then taken out and placed on a porcelain plate to dry. When the tubers were dried, each lot was introduced into a covered dish which was previously sterilized in the autoclave at 130°C. for 30 minutes ; the tubers were then sprinkled with an emulsion of potato rot organism No. 1. For a week there was no rotting. After 8 days the tubers in lots 1 and 2 began to show signs of rot and were completely rotten in about 4 or 5 days more. Those in 3a and 3b remained unaffected. It is clear then that sterilization of the skin with mercuric chloride or formalin solutions of the above strengths is not effective for preventing rot in potato tubers which are in contact with a sufficient number of rot producing organisms, although it secures them from attack for the first few days. The copper sulphate remaining on the skin of the tuber was found to be sufficient to kill the organisms introduced in the emulsion ; this was tested by making a streak on agar, which gave no growth at all from the outside skin of the tubers treated with copper sulphate.

As the potatoes in store, if kept uncovered, are subject to the attack of the potato moth, it is necessary to cover them with sand. Further

experiments were therefore made to test the efficacy of the copper sulphate treatment as a preventive of rot in tubers stored in sand.

Two experiments were made for this purpose in the year 1913. In the first, sound tubers (Naini Tal variety) grown on the Pusa farm were selected, and in the second, sound tubers selected from the various samples received for examination in this laboratory were taken. The latter had been in contact with rotting potatoes, and hence were in the natural course liable to attack, which it was sought to arrest by treatment with copper sulphate solution.

In the first experiment with sound potatoes (Naini Tal variety), the tubers were divided into two lots of twenty tubers each. One lot was treated with 2% copper sulphate solution, the second being untreated. These were again subdivided into 1A and 1B and 2A and 2B. Those in 1A and 2A were stored separately in river sand thoroughly dried in the sun and those in 1B and 2B in dry sand to which 5% distilled water was added. The moisture added was so little that it could hardly be made out by feeling with the hand. The results obtained after storing in this way for two months were as follows :—

Stored in dry sand.	Stored in moist sand.
1A.	1B.
Treated with copper sulphate solution.	Treated with copper sulphate solution.
Sound 100%. Rotten 0%.	Sound 90%. Rotten 10%.

Stored in dry sand.	Stored in moist sand.
2A.	2B.
Untreated.	Untreated.
Sound 100%. Rotten 0%.	Sound 70%. Rotten 30%.

The second experiment was carried out in exactly the same way as the first, with tubers which had been in contact with rotting tubers.

Following is the result after storing :—

Stored in dry sand.	Stored in moist sand.
Treated with Copper Sulphate solution.	Treated with Copper Sulphate solution.
Sound 100%. Rotten 0%.	Sound 80%. Rotten 20%.

Stored in dry sand.	Stored in moist sand.
Untreated.	Untreated.
Sound 70%. Rotten 30%.	Sound 0%. Rotten 100%.

As is to be seen from the above results, conditions of moisture affect the liability of tubers to rot to a very great extent. Treatment with copper sulphate is successful, but only completely so when the tubers are stored in dry sand.

Experiments on the storage of potatoes were made in the year 1914. Potatoes washed with water and then dried in the sun were taken and some of these were punctured with a sterile needle. These were then stored in dry and moist soil and dry and moist sand separately. These were stored on 20th January 1914 in glass jars and taken out on 27th August 1914. Varieties used in the experiment were Naini Tal and local Pusa bazaar potatoes.

The following is the result :—

	Punctured.	*Not punctured.*
Moist soil (10% moisture).	Completely rotten.	All germinated, but 25% were dried up and dead.
Dry soil.	Fungus. Dry rot. (*Fusarium Solan.*) Inside of potato almost completely destroyed.	100% quite sound.
Moist sand. (10% moisture).	Completely rotten.	Germinated, roots drying up.
Dry sand.	50% Rotten. 50% Sound.	100% quite sound.

This clearly shows the effect of moisture, whether on sound or punctured potatoes.

Another experiment with treatment of 2% $CuSO_4$ for 30 minutes, and after drying keeping in sand for seven months, showed the following result :—

Moist sand	..	..	50% remained sound.
Dry sand	..	..	100% remained sound.

One fact to be noticed in connection with the destruction of sound potatoes in moist soil was that they germinated some time before rotting, that the sprout was blackened first, and the rot then spread throughout the tuber.

As a result of these experiments therefore, it is clear that in storing potatoes moisture is to be avoided as far as possible. The potatoes to be stored should be dipped in copper sulphate solution and then dried in the sun ; afterwards they may be put in dry gunny bags or tied into bundles of dry grass as seed is sometimes stored in grass bundles. These should then be covered by sun-dried sand. In order to reduce access of moisture from the air a layer of dry straw or grass may be interposed between two layers of sand ; Plate III, fig. 2, shows the effect of such an intercalated stratum in restricting the movement of water through the covering layer of sand.

Care should be taken to store only sound potatoes. Tubers showing any signs of disease should not be included among those to be stored. Those injured in harvesting should be stored separately. If it be impossible to pick the injured ones from a large store, at least there should be a separate heap for storing seed potatoes, in which only picked tubers should be stored. The temperature of the godown should be as low as possible, and adequate ventilation should be secured.

It was found that no preventive treatment with antiseptics nor use of dry sand will prevent infection from eventually passing from a rotten tuber to a sound one actually in contact with it.

The rotten tuber, when it reaches an advanced stage of putrefaction, not only provides an overwhelming number of rot-producing bacteria in a highly virulent condition, but as a consequence of its decay supplies the moisture necessary for their further multiplication and activity.

The copper sulphate treatment appears to delay this transfer of infection, which will no doubt result in reducing the percentage of rotted tubers in a given time, and render more efficacious the highly necessary periodical inspection which should aim at removing such sources of infection from the store.

In any case it is essential that periodical inspection should be made in order to remove any rotten tubers, which if left would inevitably infect the whole store. The use of copper sulphate may help to delay the spread of infection from such centres, which would also be partially arrested by proper spacing amongst the tubers themselves ; this however would not be practicable on any large scale, although it might be made use of by individuals with advantage so as to minimize chances of infection by contact.

Stoward,[1] as the result of experiments, concludes that the skin of the potato is of considerable importance as offering resistance to invasion by fungi and bacteria. This skin includes an external layer of cork cells which

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[1] *Loc. cit.*

are highly resistant to such invasion, but in digging up and handling the tubers the skin is liable to mechanical injury resulting in solutions of continuity, thus affording points of ingress for bacteria and fungi.

It will be seen from photographs of sections taken radially through the eye of a potato tuber (Plate II), that the cork layer terminates at the margin of the eye, which thus constitutes a weak spot in the resistance to bacterial invasion. In treating the tubers with copper sulphate as recommended, particular care should be taken to remove all earth and dirt from the eyes by washing, as well as from the rest of the surface, so as to allow the copper salt to act on the epidermal cells of the eye and raise their resistance, which appears to result from chemical action of this reagent upon the cell tissues. From reference to Plate II, fig. 1, the importance of preventing sprouting of the tubers will be realized, as the breaking off of a sprout by handling would leave a large wound surface of tissue easily attacked by fungi or bacteria ; it is also probable that the sprout itself is more readily penetrated by fungal hyphæ or by bacteria than the cork skin of the tuber.

SUMMARY.

1. Four species of bacteria are found to be directly causative of rotting of potato tubers in India. Out of these four species the one described as organism No. 1 in this memoir was invariably present in the rotting tubers sent for examination.

2. Infection takes place from outside and not from the plant, differing in this respect from that produced by *B. solanacearum*.

3. Great care must be taken to avoid mechanical injury to tubers while digging and in handling them in transit to storage godowns.

4. Dryness is an equally essential condition to prevent bacterial invasion. Sand or any other material used for storing must be thoroughly dried before using it for storage ; sandy soil must not be substituted for sand, and this latter should be the coarsest procurable.

5. Bacterial invasion is arrested by treatment of the outside skin with 2% copper sulphate solution and drying the tubers before storing, but periodical inspection of the stored potatoes is necessary to prevent infection passing from rotting to sound tubers.

PUSA :
March 5th, 1915.

Fig. 1.
Potato tuber rotted by
P. r. Bacterium No. 1.

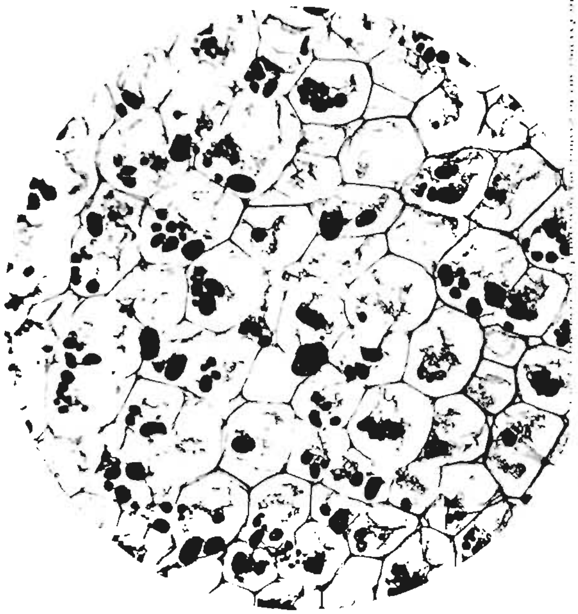


Fig. 2.
Section Normal tuber × 100. 16 mm. Zeiss Ap

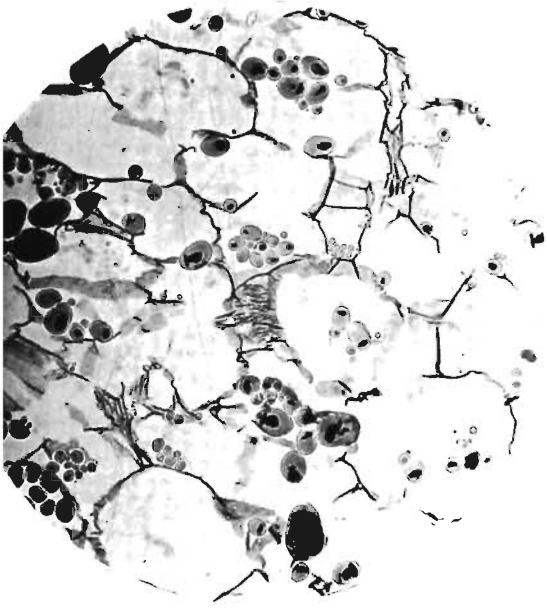


Fig. 3.
Early stage.

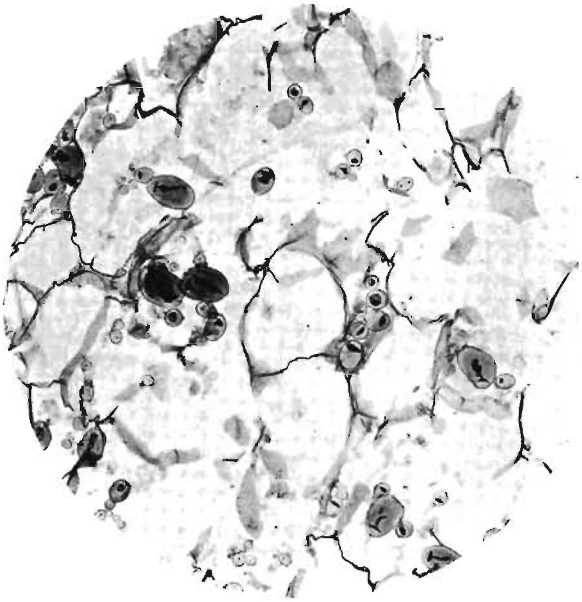


Fig. 4.
Advanced stage.

Section of rotting tuber showing breaking down of cell walls and starch grains.

Fig. 1. Section of Sprout showing cork layer.

Fig. 2. Section through "eye" of tuber.

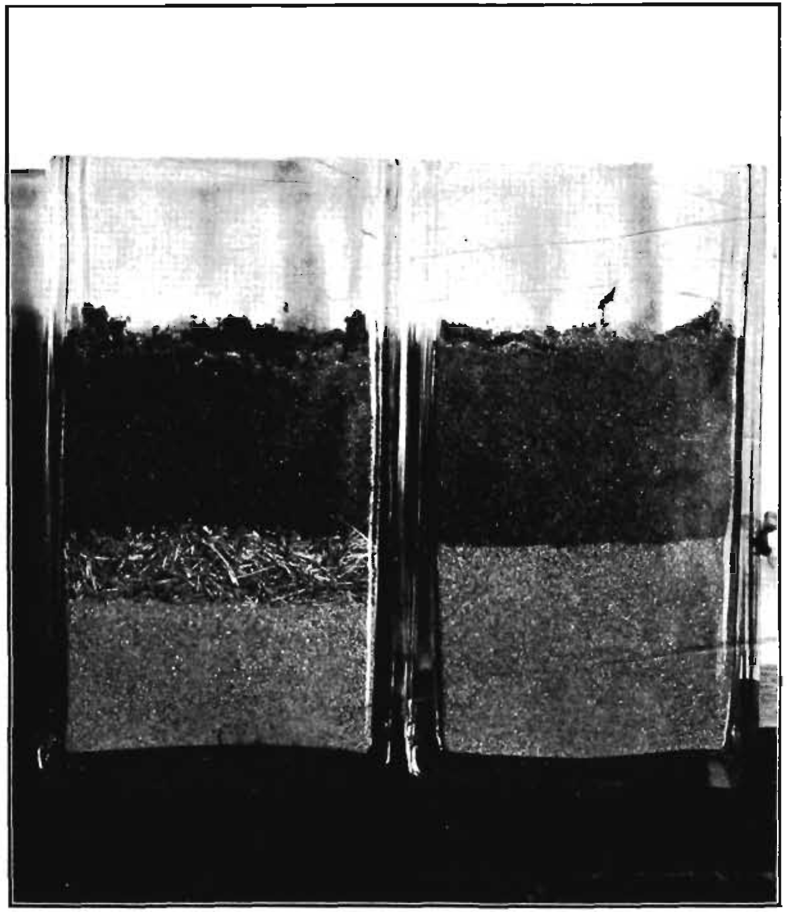


Fig. 1.

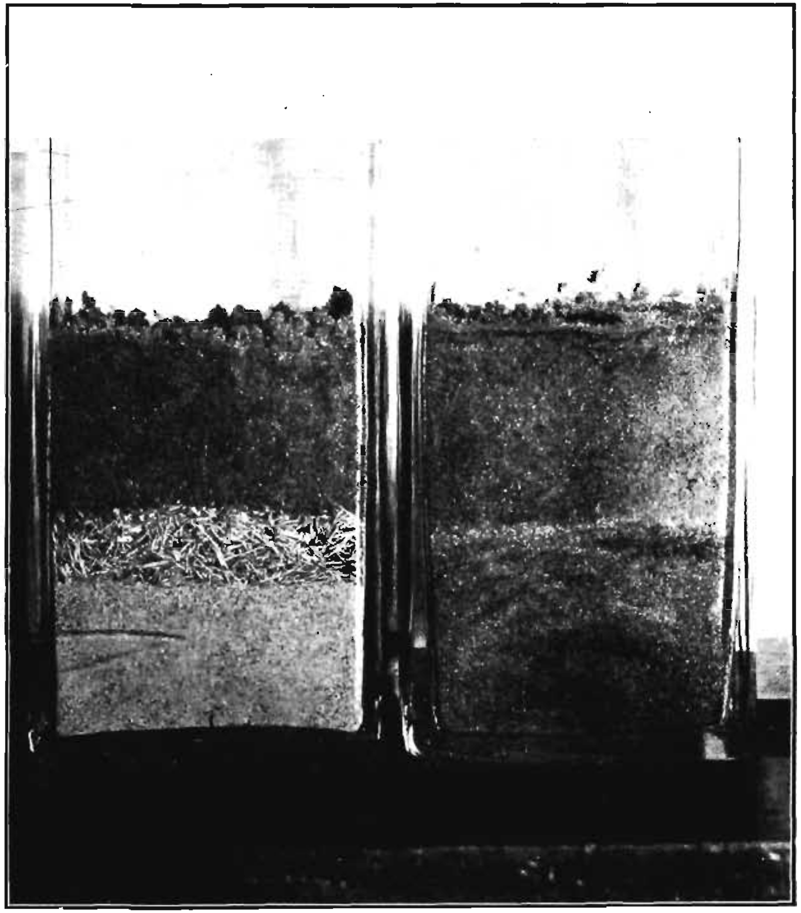


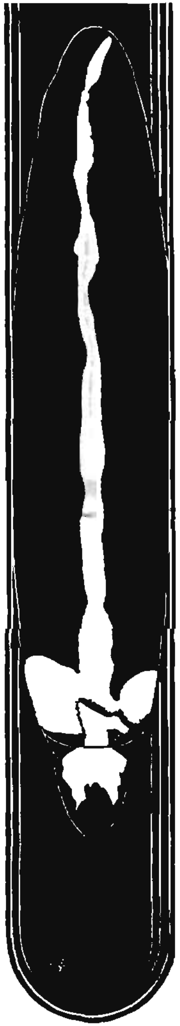
Fig. 2.

Arrest of movement of water through sand by intervening layer of grass. Upper half of sand saturated; Fig. 2 photographed 3 days later.

PLATE IV.

No. 1.
Potato 24 hours

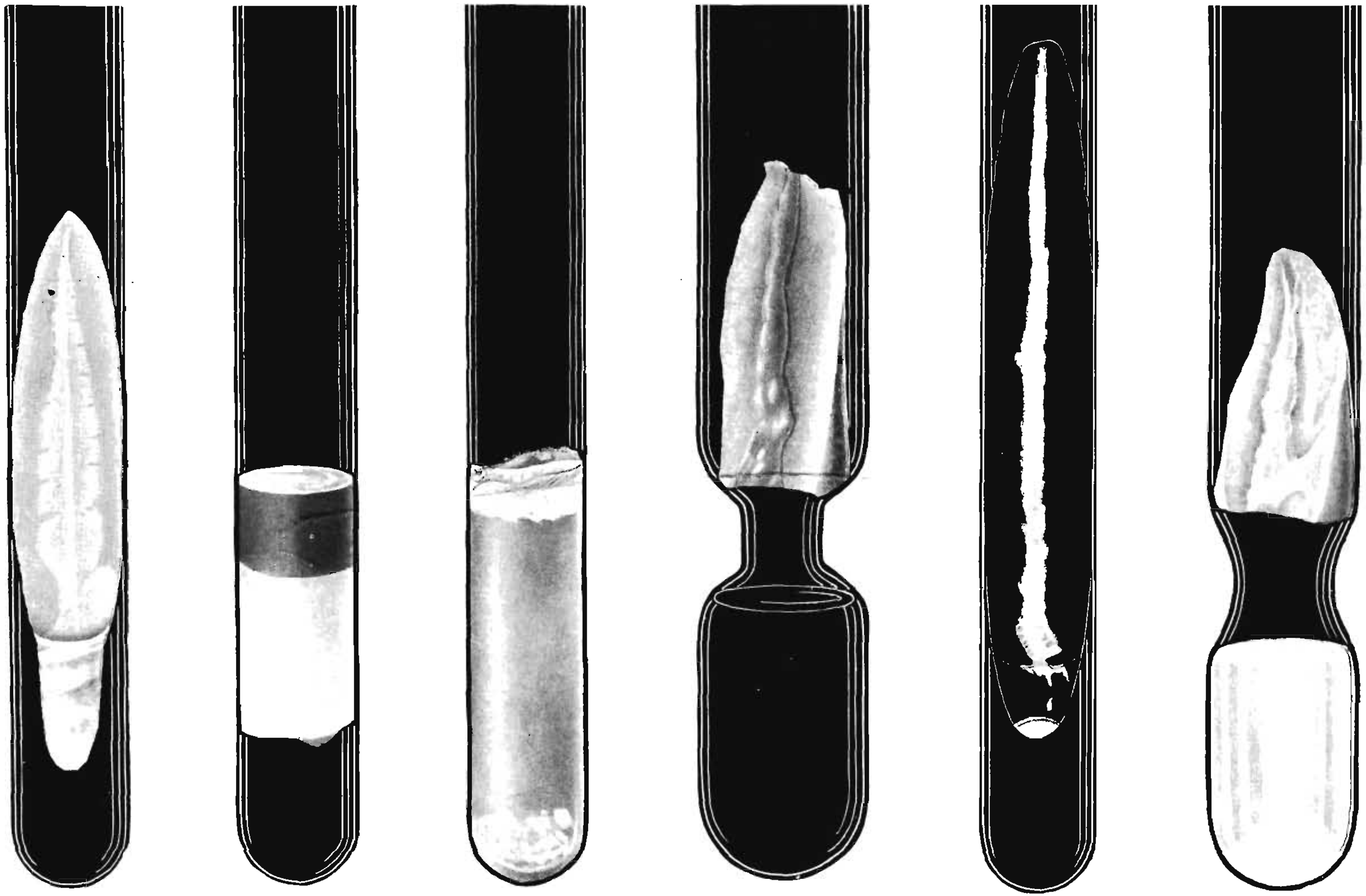
No. 2. Agar.
24 hours



No. 2. Agar.
48 hours.

No. 2. Agar.
One week.

No. 2.
Potato 24 hours.



Agar 24 hours. Gelatine 4 days.
No. 4.

Litmus Milk 7 days. Potato 3 days.
No. 4.

Agar 24 hours. Potato 3 days.
No. 3.

POTATO ROT ORGANISMS.